Sesquiterpene Lactones from *Anthemis carpatica* Willd.

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Four new eudesmanolides and two new guaianolides were isolated from the aerial parts of *Anthemis carpatica* Willd. and their structures elucidated by spectral methods. In addition, seven known sesquiterpene lactones were identified.

Introduction

The genus *Anthemis* (tribe Anthemideae, family Asteraceae) comprises about 62 species distributed in Europa (Fernandes, 1976), of which 24 are found in Bulgaria (Andreev *et al.*, 1992). *Anthemis carpatica* ssp. *carpatica* Willd. (*A. orientalis* ssp. *carpatica* (Willd.) Hayek) is growing in the mountains of Southern Europe: Pyrenees, East Alps, Carpathians and Balkan Peninsula. The literature survey revealed only two publications on *A. carpatica* of Yugoslavian origin which reported numerous sesquiterpene lactones, mainly of guaiane type (Bulatovic *et al.*, 1997 and Vajs *et al.*, 2000). Continuing our chemosystematic investigations on the Bulgarian species of tribe Anthemideae (Asteraceae family), we now report the sesquiterpene lactone profile of several Bulgarian populations of *A. carpatica.*

Experimental

Plant material

The overground parts of *A. carpatica* were collected in 2001 at a different stage of plant development from two locations in Rila mountain: under peak Kalin – sample S_1 (beginning of blossoming), sample S_2 (full blossoming) and sample S_3 (mature seeds) and near Rila lakes – sample S_4 (full blossoming). Voucher specimens (SOM Co 593–596) were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

Extraction and isolation

The air dried plant material of each sample S_1 (51 g), S_2 (9 g), S_3 (5 g) and S_4 (30 g) was extracted exhaustively at room temperature with $CHCl_3$ to give, after evaporation of the solvent *in vacuo*, the crude extracts E_1–E_4 in amounts of 7.5 g, 0.4 g, 0.3 g and 2.1 g, respectively. These extracts were worked up as described previously (Ognyanov and Todorova, 1983) and the lactone fractions L_1–L_4 (750 mg, 290 mg. 150 mg and 790 mg, respectively) were subjected to TLC comparison. Only fractions L_1 and L_3 were separated by column chromatography on silica gel using solvent mixture $CHCl_3$/MeOH with increasing polarity, and selected lactone containing subfractions (IR control) were further purified. Thus, fraction L_1 yielded: **1** (2.0 mg), **2** (18 mg), **3** (2.8 mg), **4** and **5** (3.9 mg mixture) and **6** (60 mg). The fraction L_3 afforded: **7** (5.0 mg), **8** (6.5 mg), **9** and **10** (27 mg mixture) **11** (8.0 mg), **12** (1.5 mg) and **13** (2.5 mg). Compounds **1–6** were proven to be present in L_3 by TLC comparison with the isolated lactones as standards.

8α-Isobutyryloxydouglanin (**3**)

Colourless oil, EIMS (70 eV) *m/z* (rel. int.): 334 $[M]^+$ (1), 246 $[M\text{-}C_3H_7COOH]^+$ (28), 231 $[246\text{-}CH_3]^+$ (10), 228 $[246\text{-}H_2O]^+$ (100), 213 $[228\text{-}CH_3]^+$ (81), 199 $[228\text{-}C_2H_5]^+$ (32). ^1H NMR: in Table I.

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8α-Tigloyloxydouglanin and *8α-angeloyloxydouglanin* (**4** and **5**)

Colourless oil, EIMS (70 eV) *m/z* (rel. int.): 346 $[M]^+$ (1), 246 $[M\text{-}C_4H_7COOH]^+$ (33), 231 $[246\text{-}CH_3]^+$ (8), 228 $[246\text{-}H_2O]^+$ (100), 213 $[228\text{-}CH_3]^+$ (76), 199 $[228\text{-}C_2H_5]^+$ (27). ^{1}H NMR: in Table I.

8α-Hydroxydouglanin (**6**)

Colourless crystals, m.p. 191–193 °C (Et_2O/$CHCl_3$), EIMS (70 eV) *m/z* (rel. int.): 264 $[M]^+$ (2), 246 $[M\text{-}H_2O]^+$ (93), 231 $[246\text{-}CH_3]^+$ (18), 228 $[246\text{-}H_2O]^+$ (47), 217 $[246\text{-}C_2H_5]^+$ (9), 213 $[228\text{-}CH_3]^+$ (64), 202 $[231\text{-}C_2H_5]^+$ (100). ^{1}H NMR: in Table I.

8-O-Tigloyl-9α-acetoxycumambrin B (**11**)

Oil, EIMS (70 V) *m/z* (rel. int.): 404 $[M]^+$ (0.5), 304 $[M\text{-}C_4H_7COOH]^+$ (1), 244 $[304\text{-}CH_3COOH]^+$ (14), 226 $[244\text{-}H_2O]^+$ (74), 211 $[226\text{-}CH_3]^+$ (26), 201 $[244-43]^+$ (14), 183 (10), 165 (10), 83 (100). ^{1}H NMR: in Table II.

10α-Hydroxy-9α-acetoxy-guaia-3,11(13)-dien-12,6-olide (**13**)

Oil, EIMS (70 V) *m/z* (rel. int.): 306 $[M]^+$ (0.5), 288 $[M\text{-}H_2O]^+$ (5), 246 $[M\text{-} CH_3COOH]^+$ (12), 228 $[288\text{-}CH_3COOH]^+$ (100), 213 $[228\text{-}CH_3]^+$ (46), 185 (28), 149 (63), 133 (35). ^{1}H NMR: in Table II.

Results and Disscusion

Four taxa of *A. carpatica* collected in south-west Bulgaria were subject of the present study. The air-dried plant material was extracted with chloroform and worked up to give the corresponding lactone fractions L_1–L_4. TLC comparison revealed that fractions L_1, L_2 and L_4 were identical regarding the main constituents but fraction L_3 contained much more spots besides those visible in the other three samples. For that reason, fractions L_1 and L_3 were only subjected to chromatographic separation (see Experimental). Thus, six sesquiterpene lactones were isolated from L_1, two of which were identified by analogy of their spectral data to those published as douglanin (**1**) (Matsueda and Geissman, 1967) and ludalbin (**2**) (Geissman and Saitoh, 1972). Although the spectral data of the other four lactones were very similar to those of **1** and **2**, they all were found to be new natural products. As the ^{1}H NMR spectra of **3** and the inseparable mixture of **4** and **5** (Table I) only differed in the signals typical for the ester side chains from that of ludalbin (**2**), the presence of new derivatives of douglanin (**1**) was very likely. Moreover, the similarity of the chemical shifts and couplings of H-1 and H-5/H-8 were in agreement with the same relative stereochemistry of these new lactones as that of **2**. The nature of the ester side chains was evident from the ^{1}H NMR spectra which contained signals typical of the isobutyrate, tiglate and angelate groups in **3**, **4** and **5**, respectively. This was further supported by the intensive peak at *m/z* 246, the presence of which in the mass spectra is obviously due to the loss of the corresponding aliphatic acids (see Experimental). Accordingly, the new sesquiterpene lactones were identified as 8α-isobutyryloxydouglanin (**3**), 8α-tigloyloxydouglanin (**4**) and 8α-angeloyloxydouglanin (**5**).

1 R = H
2 R = OAc
3 R = OiBu
4 R = OTig
5 R = OAng
6 R = OH

7 $R_1 = R_2$ = OAc;
8 R_1 = OAc; R_2 = OiBu
9 R_1 = OH; R_2 = OAc
10 R_1 = OAc; R_2 =OH
11 R_1 = OAc; R_2 = OTig
12 R_1 = H; R_2 = OAc
13 R_1 = OAc; R_2 = H

Fig. 1. **1**: douglanin; **2**: ludalbin; **3**: 8α-isobutyryloxydouglanin; **4**: 8α-tigloyloxy douglanin; **5**: 8α-angeloyloxydouglanin; **6**: 8α-hydroxydouglanin; **7**: 9α-acetoxy cumambrin A; **8**: 8-O-isobutyryl-9α-acetoxycumambrin B; **9**: 9α-hydroxy cumambrin A; **10**: 9α-acetoxycumambrin B; **11**: 8-O-tigloyl-9α-acetoxycumambrin B; **12**: cumambrin A, **13**: 10α-hydroxy-9α-acetoxy-guaia-3,11(13)-dien-12,6-olide.

The structure of **6**, molecular formula $C_{15}H_{20}O_4$, could be also readily deduced from the ^{1}H NMR spectrum (Table I). It was very close to those of the eudesmanolides **2**–**5**, only the H-8 signal being shifted upfield to δ 4.14 and the signals for the ester groups being absent. The MS of **6** displayed, along with the molecular peak at *m/z* 264, two fragments at *m/z* 246 and *m/z* 228 which required the presence of two hydroxyl groups. Their loca-

Table I. ^{1}H NMR data of compounds **3–6** in CDCl$_3$ (250 MHz).

H	**3**	**4** and **5**	**6**
1	3.43 brd (3.9)	3.43 brd (3.9)	3.38 brt (4.3)
2	2.05 brd (19.3)	2.05 brd (19.3)	2.02 brd (18.1)*
2′	2.50 brd (19.3)	2.50 brd (19.3)	2.40 brd (18.1)
3	5.32 brs	5.32 brs	5.28 brs
5	2.72 brd (11.7)	2.74 brd (11.7)	2.73 brd (11.8)
6	4.06 dd (10.9, 11.7)	4.08 dd (10.9, 11.7)	4.05 dd (10.7, 11.8)
7	2.86 dddd (10.9, 10.9, 3.1, 2.9)	2.90 dddd (10.9, 10.9, 3.1, 2.9)	2.52 dddd (10.7, 10.7, 3.2, 3.1)
8	5.26 ddd (5.2, 10.1, 10.9)	5.28 ddd (5.2, 10.1, 10.9)	4.14 ddd (5.1, 10.0, 10.7)
9	ca. 2.05 m	ca. 2.05 m	ca. 2.02 m*
9′	1.84 dd (5.2, 12.5)	–	1.58 dd (4.3, 12.6)
13	6.12 d (3.1)	6.13 d (3.1)	5.96 dd (1.4, 3.2)
13′	5.52 d (2.9)	5.53 d (2.9)	5.94 dd (1.4, 3.1)
14	0.91 s	0.93 s / 0.94 s	0.85 s
15	1.89 brs	1.89 brs	1.81 brs
OH	–	–	3.95 d (4.3)
OR	i-But	Tig / Ang	

i-But: 2.59 qq (7.0), 1.19 d (7.0), 1.20 d (7.0); Tig: 6.91 qq (7.0, 1.3), 1.82 dq (7.0, 1.3), 1.87 quint (1.3); Ang: 6.17 qq (7.0, 1.5), 1.89 dq (7.0, 1.5), 1.87 quint (1.5).
* Overlapping signals.

tion at C-1 and C-8, and relative stereochemistry followed from the observed couplings of H-1 and H-8, respectively. These spectral features are again reminescent of douglanin (**1**) and suggested the lactone **6** to be 8α-hydroxydouglanin.

The lactone fraction L$_3$ afforded, in addition to the eudesmanolides described above, seven further sesquiterpene lactones which proved to be of guaiane type. On the basis of the identity of the observed spectral data with those reported in the literature, five of the isolated lactones were identified as 9α-acetoxycumambrin A (**7**) (Bulatovic *et al.*, 1997), 8-O-isobutyryl-9α-acetoxycumambrin B (**8**) (Vajs *et al.*, 2000), 9α-hydroxycumambrin A (**9**) (Bulatovic *et al.*, 1997), 9α-acetoxycumambrin B (**10**) (Bulatovic *et al.*, 1997), and cumambrin A (**12**) (Irwin *et al.*, 1969).

Lactone **11**, a colourless gum had a molecular formula of $C_{22}H_{28}O_7$ (*m/z* 404, M$^+$). The mass spectral fragmentation pattern suggested the presence of two ester groups (*m/z* 304 [M-100]$^+$, *m/z* 244 [304–60]$^+$) and a hydroxyl group (*m/z* 226 [244–18]$^+$). The ^{1}H NMR spectrum at room temperature showed considerable broadening of almost all the signals obviously due to a conformational exchange (Table II). However, most of the signals split into pairs of sharp, well-resolved resonances at low temperature (−55 °C), thus indicating the existence of two conformers, similar to those described for 8-O-isobutyryl-9α-acetoxycumambrin B (Vajs *et al.*, 2000). The ^{1}H NMR data presented in Table II are consistent with a guaiane skeleton bearing a 12,6-trans annelated α-methylene-γ-lactone ring, a double bond at C-3/C-4, a

Table II. ^{1}H NMR data of compounds **11** and **13** in CDCL$_3$ (250 MHz).

H	**11** (room t°)	**11** (−55° C)	**13**
1	2.87 br*	2.80*/3.11*	2.88 m*
3	5.52 brs*	5.52 brs	5.50 brs
5	2.87 br*	2.80* / 3.11*	2.88 m*
6	4.23 brt (9.8)	4.26 t (9.8) / 4.40 t (9.5)	4.15 t (10.0)
7	3.75 m	3.95 dddd (9.8, 9.2, 3.5, 3.2) / 3.53 dddd (9.5, 9.3, 3.2, 2.5)	3.18 m
8	5.52 brs*	5.65 dd (9.2, 5.2)/5.31 dd* (9.3, 4.1)	
9	5.32 d (4.2)	5.32 d* (5.2) / 5.35 d* (4.1)	5.13 dd (4.5; 7.0)
13	6.19 d (3.5)	6.21 d (3.5) / 6.34 d (3.2)	6.22 d (3.5)
13′	5.52 brs*	5.43 d (3.2) / 5.84 d (2.5)	5.46 d (3.2)
14	1.22 s	1.23 s / 1.26 s	1.17 s
15	1.87 brs	1.90 brs / 1.84 brs	1.86 brs
OAc	2.07s	2.04 s / 2.25 s	2.15 s
OTigl	1.86dq (7.0, 1.3)	1.90 brd (7.0)	
	1.90 dq (1.3, 1.3)	1.92 brs	
	6.90 qq (1.3, 7.0)	6.98 qq (7.0, 1.3) / 6.91 qq (7.0, 1.3)	

* Overlapping signals.

methyl geminal to a hydroxyl group and two ester groups – an acetoxy and a tigloyloxy group (Table II). Their location at C-8 and C-9, respectively, followed from the coupling patterns of the adjacent protons H-8 (dd) and H-9 (d), along with the NOEs observed between H-13′ and the tigloyl protons H-3′ and H-5′. Furthermore, the magnitude of the coupling constants of H-6 – H-9 and the NOEs between the hydrogen pairs H-6/H-8, H-6/H-14 and H-9/H-14 were in full agreement with the *syn*-β-orientation of H-6, H-8, H-9 and the C-10 methyl group. Hence, **11** was identified as 8-O-tigloyl-9α-acetoxycumambrin B.

The structure of compound **13** also followed from the ^{1}H NMR data (Table II) which were very similar to those of **12**. This was not unexpected, as the two guaianolides differed in the position of the acetate ester group only. The placement of the latter at C-9 and its α-orientation was based on the multiplicity of the H-9 signal (dd, J = 4.5, 7.0 Hz) and the observed upfield shift of H-7 ($\Delta\delta$ = 0.71 ppm) and downfield shift of H-1 ($\Delta\delta$ = 0.32 ppm) in comparison to the chemical shift of the same protons in **12**. Moreover, the ^{1}H NMR data were in a good accordance with those reported for 10α-hydroxy-9α-propionyloxy-guaia-3,11(13)-dien-12,6α-olide (Zdero *et al.*, 1990). Thus, lactone **13** was proven to be 10α-hydroxy-9α-acetoxy-guaia-3,11(13)-dien-12,6-olide.

The results described above show unambiguously that the lactone composition depends on the stage of the plant development. Thus, the lactone profile of the samples S_1 (beginning of blossoming), S_2 and S_4 (full blossoming) was the same and eudesmanolides were the only type of compounds. However, along with these lactones, seven guaianolides were isolated from sample S_3 (mature seeds). The formation of guaianolides, in addition to eudesmanolides in a more advanced phenophase of the species suggests that the observed chemical difference is a result of active seasonal plant changes.

It should be noted that the eudesmanolides which are found to be the main lactones in the studied taxa are not typical constituents of *Anthemis* species. As a matter of fact, douglanin is the only eudesmanolide found so far in this genus (El-Alfy *et al.*, 1989). Moreover, all the described eudesmanolides, except douglanin and ludalbin are new representatives of the small group of 1α-hydroxy-eudesma-3, 11(13)-dien-12,6-olides.

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